Protocol for Monitoring Incubator # 3

- Open one Tupperware container at a time. Look at each petri dish.

- If any wasps or GWSS nymphs are visible put that petri dish to one side.

- If nothing is visible tap the petri dish on its side lightly. The underside of the leaf is a great hiding spot.

- Collect the wasps and/or GWSS from one petri dish at a time.

- All insects are stored in a 95% alcohol solution. Wasps are stored in the 2ml vials with the purple lids. Nymphs are stored in either the 8ml or 5ml glass vials with black lids.

- GWSS may be gassed before collection. Try to count the number of nymphs before collecting. To gas them: put the petri dish in the white cardboard container and slightly open the petri dish lid. Let a small amount of CO2 into the container and shut the lid. Wait a few seconds and then collect the GWSS with an alcohol-wetted paintbrush. Deposit each GWSS nymph in the vial for the location it came from, making sure it is the correct year. Another method of gasing is to slightly open the petri dishes making sure that none of the nymphs escape. Gas for a few seconds then close the dish completely and wait a few seconds more.

- Record the number of GWSS nymphs collected from each petri dish on the data sheets. If any GWSS nymphs escape, record this information on the on the data sheet.

- For each petri dish that contains wasps a new vial will have to be labeled. Each leaf has a vial code that is a letter for the month, and a number for the order it was collected in. Write vial number, the site code, and the date on the lid of the vial

- Record the number of wasps collected from each petri dish on the data sheets. If any wasps escape, record this information on the data sheet. Do not kill *G. morgani*. Also do not kill any species that you do not recognize without consulting David or Jessica.

- If you find more than one species per dish place them in separate vials labeled with the above info. In addition, you can distinguish the vials by adding a “b”

to the vial code or writing the species on the lid. Make sure you note which species in which vial on the data sheet.

- If any of the cotton pads are too dry, add a few drops of the water to each. If they are too wet, shake off excess water. Wipe off any excess condensation that formed on the lid of the petri dishes.

- Repeat for all Tupperware containers in incubator 3.

Additional notes for checking the monitoring in Inc #3:

- The main types of wasps that we find are *G. triguttatus, G. ashmeadi, G. morrilli/G. walkerjonesi, and Ufens* (trichogrammatids). *G. morgani* is a fairly new species usually found in Orange and San Diego counties.

- *G. ash* is found mainly in the interior sites. The *G. morrilli/G. walkerjones* species are mostly found in the coastal areas. *Ufens* are found mostly at the desert sites (RCOD1, the Imperial county sites), and also at BCRA1.

- *Ufens* are very small. More then one *Ufens* can emerge from the same egg. The number of *Ufens* collected from one petri dish should match the number of exit holes. Sometimes they can escape out of the petri dishes. You should check the inside of the Tupperware container if you suspect this has happened.

- *Ufens* can also take longer to emerge. You can keep the Tupperware containers from the sites listed above up to a week longer if you suspect more wasps may emerge.

- If you are not sure of a species of wasp just label it as unknown. I can always check them later or send them off to be identified.

- The petri dishes are kept in the incubator and observed daily for approximately 2 weeks from the date of collection, except where noted above, or if you suspect that you might still get emergence from an egg mass.

- At the end of the observation period, the mold condition of leaf (on a 0 to 5 scale) is recorded in the mold column of the data sheet. Then you can open the egg mass with a probe and using a magnifying lens or microscope record any corpses of insects that did not emerge. Record this in the # left column. If unable to tell you can put a question mark.